D-25799- A - TFM

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	JEAN-CLAUDE BYSTRYN	
Serial No.	:	485,780	Group Art Unit 186
Filed	:	February 22, 1990	Examiner J. Kushan
For	:	ANTI-CANCER VACCINE	

D E C L A R A T I O N

JEAN-CLAUDE BYSTRYN declares and states:

(1) He is the Jean-Claude Bystryn who is the applicant of the above-identified application Serial No. 485,780 filed February 22,1990, which is a file wrapper continuation of Serial No. 41,864 filed April 23, 1987, now abandoned;

(2) He has read and understood the application as filed including the newly submitted claims and he has read and understood the Declaration and Power of Attorney which accompanied the parent application Serial No. 41,864 as filed; in said Declaration and Power of Attorney he stated he is the original, first and sole inventor of the subject matter thereof, the invention for which a patent is sought, based on the subject application;

(3) He affirms and confirms that he is the original, first and sole inventor of the invention disclosed and claimed in the subject application and in the parent application Serial No. 41,864 including the newly submitted claims forwarded herewith along with this Declaration;

(4) He is familiar with, as co-author, of the paper entitled "Cellular Immune Response to Melanoma Antigen Vaccine". Clinical Research "A", May 5, 1986, copy attached, and which is cited as a reference by the Examiner in connection with the prosecution of his above-identified patent application. This publication lists him as co-author with R. Oratz, M. Harris, D. Roses and J. Speyer. Doctors Oratz, Harris, Roses and Speyer are oncologists and surgeons who had no involvement in the development of the subject invention and the claimed vaccine and its preparation. The only involvement of the co-authors, Drs. Oratz, Harris and Speyer and the reason for their listing as authors, was they provided patients for the vaccine studies and, in the case of Dr. Oratz, assisted in treating patients with vaccines. He, applicant herein, Jean-Claude Bystryn, again reaffirms and confirms that none of the above-identified co-authors along with him in the above-identified paper inventively contributed to his invention as disclosed and claimed in the above-identified application and in the newly submitted claims,

as to be co-inventor with him and that he is the original, first and sole inventor of the invention disclosed and claimed in the above-identified appliation.

He hereby declares that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

JEAN-CLAUDE BYSTRYN

Dated: 10.18.010

Program—Poster Session

Jointly Sponsored by the

THE ASSOCIATION OF AMERICAN PHYSICIANS

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THE AMERICAN FEDERATION FOR CLINICAL RESEARCH

The Sheraton Washington Hotel
Washington, D.C.

MONDAY, MAY 5, 1986

POSTER SESSION
12:00 Noon - 1:30 PM
Sheraton Hotel
Exhibit Hall B

Allergy
Cardiovascular
Clinical Epidemiology and Health Care Research
Clinical Pharmacology
Endocrinology
Gastroenterology
Genetics
Hematology
Hypertension
Immunology
Infectious Disease
Metabolism
Pulmonary
Renal and Electrolyte
Rheumatology

Members of the Societies are urged to visit frequently exhibits that provide valuable support for this meeting. The exhibits are located in The Sheraton Washington Hotel, Exhibit Hall A, Friday, 7:30 - 9:30 PM; Saturday and Sunday, 9:30 - 4:00 PM; and Monday, 9:30 - 1:30 PM.

Forty-Third Annual Meeting

THE AMERICAN FEDERATION FOR CLINICAL RESEARCH

Sheraton Washington Ballroom
Washington, D.C.

MONDAY, MAY 5, 1986

PLENARY SESSION, 8:00 AM

General Business Meeting
Edwin C. Cadman and Gary W. Hunninghake, *Presiding*

AFCR AWARD FOR CLINICAL RESEARCH
(Supported by Burroughs Wellcome Fund)

Barton F. Haynes
Duke University School of Medicine
The Role of the Thymic Microenvironment in Promotion of Early Stages of Human T Cell Maturation

PRESIDENTIAL ADDRESS
Edwin C. Cadman

Intermission, 9:15 - 9:30 AM

FRONTIERS IN CLINICAL SCIENCE SYMPOSIUM

Role of Prions in Degenerative Neurological Disease
Stanley B. Prusiner
University of California School of Medicine

Cachectin—The Dark Side of TNF
Anthony Cerami
Rockefeller University

Angiogenesis and Angiogenesis Factors
M. Judah Folkman
Harvard Medical School

Acute Myocardial Infarction: Reperfusion and Reflow Injury
Myron L. Weisfeldt
Johns Hopkins Hospital

R1 04257994 **AFCR ONCOLOGY**

CELLULAR IMMUNE RESPONSE TO A MELANOMA ANTIGEN VACCINE. JC Bystryn, R Oratz*, N Harris*, D Rosen*, J Speyer*. NYU School of Medicine and Kaplan Cancer Center, NYC, NY, USA.

This study was conducted to examine methods of enhancing the immunogenicity of a tumor antigen vaccine in man. A polyvalent melanoma associated antigen (MAA) vaccine was prepared from material shed by pooled allogeneic melanoma cells. It contained multiple MAAs, and was free of detectable fetal calf serum proteins and Dr antigens. The effect of three different methods of immunization was studied in 36 patients with stage II disease. Ten pts were immunized weekly with escalating doses of vaccine (protocol I), 9 pts were immunized every two weeks with a fixed dose (protocol II), and 17 pts were immunized similarly to protocol II but with alum as an adjuvant. In addition we examined the immunopotentiating effect of low dose cyclophosphamide (300 mg/ml, 3 days prior to each immunization) in patients with stage III melanoma. All immunizations were given intradermally into all 4 extremities for 2 months. Cellular immunity to melanoma was assessed by delayed cutaneous hypersensitivity (DCTH) responses to vaccine prior to and after 2 months of immunization. DCTH was induced or augmented in 20%, 67%, 71% of stage II patients immunized on Protocols I, II and III respectively. Skin tests of positive patients with an equal amount of control vaccine prepared from pooled allogeneic peripheral leukocytes were negative, indicating that the reaction to the vaccine was selectively directed to melanoma. Pre-treatment with cyclophosphamide induced or augmented DCTH in 67% of 6 stage III patients. These results indicate that immunization to a melanoma vaccine can enhance cellular immune reponse to melanoma. Bi-weekly immunizations seem to be more immunogenic than weekly immunizations, and alum or pre-treatment with low dose cyclophosphamide does not significantly augment immunogenicity.

IN VIVO ANTI-TUMOR EFFECTS OF T101+DOX PRESENCE OF ANTIGENIC MODULATION. Shawler*, and DE Johnson*. VAMC & U.C Medicine & Cancer Center, San Diego, CA

We examined the anti-tumor effe monoclonal antibody T101+Doxorubicin setting of antigenic modulation in a MOLT-4 tumors (Dillman et al, Cance 1985). Previous experiments demonst anti-tumor effects for T101+DOX as alone, T101 alone, or a T101-DOX-de While there appeared to be a synergisti T101 & DOX, there was also evide immunoreactive T101-DOX complex which specific cytotoxicity. In these pretreated tumor bearing animals with produced sustained antigenic modulat days. Animals were given phosphate (PBS), DOX, more T101, or T101+DOX PBS+PBS injections were used as a neg PBS followed by T101+DOX as a positiv were no tumor regressions in animals re T101+PBS, or T101+T101. There were 7 PBS+T101+DOX, 4 with T101+Dox, & 2 wit Growth curve analysis confirmed supe T101+DOX. We conclude that pre-exi modulation does significantly impede effects of T101+DOX, but there m synergistic effect between T101 & DOX.

DNA DAMAGE PRODUCED BY AMSACRINE AND RELATED ACRIDINES IN L1210 CELLS AND ISOLATED NUCLEI. JM Covey,* YG Pommier, and KW Kohn*, Laboratory of Molecular Pharmacology, DTP, DCT, NCI, NIH, Bethesda, MD.

Amsacrine (m-AMSA; NSC 249992) is a DNA binding 9-aminoacridine with demonstrated clinical activity against acute leukemias. We have investigated the effects of m-AMSA and several derivatives (A, NSC 343499; B, SN16507; C, NSC 140701; D, SN13553) on DNA integrity in L1210 leukemia cells and isolated nuclei. These compounds bind to DNA with varying affinities and sequence selectivities, and have been shown to trap topoisomerase II-DNA complexes in vitro, resulting in protein-associated single (SSB) and double strand breaks in DNA. L1210 cells or isolated nuclei were treated with the various acridines (0.1-50 μM) for 0.5 to 1 hr and subsequently analyzed using the alkaline elution technique. DNA-protein crosslinks (DPC) were produced in cells (6000-60000 rad eq.) by m-AMSA, and A-C, but D was inactive. At 1 μM, potency was in the order C>m-AMSA>B>A>D. DPC were also produced in nuclei, but differences between compounds were less pronounced than in cells. D caused extensive DPC in nuclei, suggesting that it enters cells poorly. Levels of DPC and SSB in nuclei were similar for A, but C produced SSB in 1.5 to 3x excess over the level of DPC. SSB were reduced below control levels in nuclei treated with D, indicating a more complex activity for this compound. Colony-formation assays demonstrated a 1-2 log cell kill with activities in the order C>B>A=m-AMSA>D. Correlation of DPC with cell kill gave similar log-linear relationships for A, B, & C, but m-AMSA had a lower slope (less kill/DPC) than the other compounds. These results indicate that the acridine derivatives studied share many of the actions of m-AMSA, but vary in potency, and may produce additional DNA damage by mechanisms unrelated to topoisomerase II trapping.

ONCOGENE ACTIVATION OF MEMBRANE PROTEASES: RO John F. DiStefano*, Cindy Anne Cotto, Bernard Northport, N. Y. and SUNY, Stony Brook, N. Y.

Using the tumor induced RBC cytolysis assa of cancer cell membrane proteases in normal ce shown that the SRC gene from Rous Sarcoma Viru the activity of a cytolytic, leupeptin inhibit protease in both chick fibroblasts and transfe press, Cancer Research). To clarify the role protease activity, an electron microscopically brane fraction from the SRC-transfected 3T3 ce Using an electrophoretic gelatinase assay we h membrane proteases we have found 6 plasminogen membrane proteases with M.W.'s in the range of Using 32 P-ATP to label the Triton solubilize all the high M.W. (>30,000) membrane proteins, appear to be alkali-stable phosphorylated sug phosphorylated on tyrosine by pp60 V-SRC, the Using synthetic substrate protease assays the preparation is enriched 7 times and 12 times i trypsin-like protease activity, respectively. membrane active, GTP binding, oncogene family, have also been transfected into mammalian cell sis assay the RAS oncogene appears to modulate cytolytic, plasma membrane, metallo-protease. that oncogenes may have a role in modulating c

ETOPOSIDE(VP-16) PLUS FLUOROPYRIMIDINES(FP): ENHANCED CYTOTOXICITY VIA DNA REPAIR INHIBITION IN L1210 CELLS. LR Damon and EC Cadman, Cancer Research Institute, University of California, San Francisco, CA.

There is recent evidence that FP incorporated into DNA stimulate DNA repair and initiate single strand breaks(SSB). Since VP-16 inhibits Topoisomerase II, which reseals repair nicks, we hypothesized that these drugs would produce synergistic cytotoxicity via enhancement of

CONCURRENT ADMINISTRATION OF AMPHOTERICIN B (A TRANSFUSIONS (GVHD IO): LACK OF J. Randall,* D. Papenberg,* C.A. Schiffer,** J Wiernik.** Albert Einstein Col of Medicine, M Maryland Cancer Center, Baltimore, MD.

Only 3/11 patient courses in 30 patients wi received concurrent ampho (0.6mg/kg/day) and rare